Volume 264, number 1, 121–124 FEBS 08411 May 1990

A nucleotide sequence in the translation start signal region is involved in heat shock-induced translation arrest in *Escherichia coli*

Yoshitaka Kuriki

Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan

Received 23 February 1990; revised version received 20 March 1990

In *Escherichia coli* synthesis of several proteins is transiently depressed upon heat shock treatment. A comparison of nucleotide sequences of the genes encoding these proteins revealed the occurrence of a consensus sequence, GAGGAA$(N)_{3-6}$ATG, in their translation start signal region. To examine whether this sequence is involved in heat shock-induced depression of protein synthesis, DNA segments corresponding to this region of four of these genes, *fusA*, *rpoB*, *glnS*, and *pheT*, were synthesized, and each of them was fused in frame with the *lacZ* gene on the open reading frame vector pORF1. The effect of heat shock on the synthesis of β-galactosidase encoded by these fused genes was then studied in *E. coli*. It was thus found that β-galactosidase synthesis starting from the inserted translation start signal was arrested transiently upon temperature shift-up from 30 to 42°C. I conclude that the heat shock-induced depression of gene expression is an event taking place at the initiation of translation.

Translation arrest; Translation start signal; Heat shock; Gene *amp*; Gene *fus*; Gene *rpoB*; Gene *glnS*; Gene *pheT*; *Escherichia coli*

1. INTRODUCTION

When *Escherichia coli* is exposed to heat shock, synthesis of several proteins is enhanced and that of another set of proteins is depressed transiently [1]. Although heat shock-induced stimulation of protein synthesis has been studied extensively, little is as yet known of the transient depression of protein synthesis [2]. I have recently reported that synthesis of β-lactamase encoded by the *amp* gene of pBR322 in *E. coli* ceases transiently as a result of temperature shift-up and that this arrest takes place at the translational level [3]. I have also found that an element involved in this arrest is located in the *amp* translation start signal region[1] [4,5], which is composed of a Shine-Dalgarno sequence (AAGGA) [6] and the start codon (ATG) separated by a pentanucleotide spacer (AGAGT) [7]. These findings suggest that upon heat shock treatment expression of the *amp* gene is transiently shut off at the initial step of translation. Since the genome of *E. coli* contains a number of genes such as *fusA* [8], *rpoB* [9], *glnS* [10], and *pheT* [11], expression of which is transiently depressed by heat shock, it was of interest to examine if these genes also contain, in their translation start signal regions, an element similar to that detected in the *amp* gene.

In this study, I found a sequence similarity between the translation start signal region of the *amp* gene and those of *E. coli* genes, expression of which is transiently depressed by heat shock. A consensus of the sequences of the region was found to be GAGGAA$(N)_{3-6}$ATG, where N is any nucleotide. Since this finding raised the possibility that the mechanism of heat shock-induced depression of the expression of these *E. coli* genes is similar to that operating in the expression of the *amp* gene, I examined whether the translation start signals of these genes are actually involved in the heat shock-induced arrest of gene expression, as is the case for the corresponding region of the *amp* gene.

2. MATERIALS AND METHODS

2.1. *Materials*

The following materials were obtained from the sources indicated: Restriction endonucleases (New England Biolab and Nippon Gene); T4 DNA ligase (Boehringer Mannheim); M13mp10 phage, [α-^{35}S]dCTP (3000 Ci/mmol) and DNA sequencing kit (Amersham); all reagents required for oligodeoxynucleotide synthesis (Applied Biosystems); and 5-bromo-4-chloro-3-indolyl-β-galactoside (X-Gal) (Wako Chemicals). The open reading frame vector pORF1 [12] was propagated in *E. coli* and purified as described [13].

2.2. *Medium*

L-broth [13] was used. When necessary, X-Gal dissolved in *N,N*-methylformamide (10 mg/ml) and ampicillin were added to L-broth to concentrations of 10 and 100 μg/ml, respectively.

2.3. *Synthesis of translation start signals and their insertion into pORF1*

All DNA manipulations were conducted by standard methods [13]. Oligodeoxynucleotides corresponding to the translation start signals of the *fusA*, *rpoB*, *glnS*, and *pheT* genes were synthesized according to Sinha et al. [14] in an Applied Biosystems model 380B DNA syn-

[1] Translation start signals are defined as those consisting of a Shine-Dalgarno sequence, an initiation codon, and a spacer of 3–11 nucleotides between them. The ribosome binding site of mRNA includes this signal

Correspondence address: Y. Kuriki, Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565, Japan

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

thesizer and purified by HPLC on an Aquapore column. The single stranded DNA synthesized was annealed with the complementary strand to form double stranded DNA, which was then inserted into the *Sma*I site of pORF1 by blunt end ligation with T4 DNA ligase. The constructs were introduced into *E. coli* JM109 (*recA*1 *Δ(lac-proAB) endA*1 *gyrA*96 *thi*-1 *hsdR*17 *relA*1 *supE*44/F' *traD*36 *rpoA*$^+$ *lac*^{q} *lacZ*ΔM15). For screening the transformants, the cells were grown on L-broth agarose plates containing ampicillin and X-Gal. Plasmids were isolated from the transformants showing the Ap^r and LacZ$^+$ phenotypes and characterized by restriction mapping and by sequencing of the fusion joints by the method of Sanger et al. [15].

2.4. *β-Galactosidase assay*

β-Galactosidase produced in *E. coli* by direction of the constructed plasmids was assayed by the method of Miller [16]. One unit of activity is defined as an increase in absorbance of 0.001 at 420 nm per min at 28°C.

3. RESULTS AND DISCUSSION

Table I shows *E. coli* genes, expression of which has been reported to be transiently depressed by heat shock [1]. The nucleotide sequence of the translation start signal region is known for 12 out of the 17 genes listed. A comparison of these sequences and that of the corresponding region of the *amp* gene indicates that they are significantly similar to one another with GAGGAA(N)$_{3-6}$ATG as a consensus. In this consensus sequence, the 5'-terminal G is followed by a Shine-Dalgarno sequence (AGGA), which is separated from the initiation codon (ATG) at the 3'-terminus by a spacer consisting of 4–7 nucleotides. The 5'-terminus of the spacer is A, but the rest of it can be any nucleotides.

Since the translation start signal of the *amp* gene has been shown to be responsible for heat shock-induced arrest of expression of this gene [4,5], it was likely that the corresponding regions of the *E. coli* genes listed in Table I are also involved in the heat shock response. This possibility was tested with the aid of the open reading frame vector pORF1 [12], as was successfully done in analyzing the involvement of the *amp* translation start signal in the heat shock response [4,5]. For this purpose, oligodeoxynucleotides corresponding to the translation start signals of the *fusA, rpoB, glnS,* and *pheT* genes were designed and synthesized (Table II). The translation start signal of the *amp* gene, which had been previously synthesized [4], was also used. Each of these oligodeoxynucleotides was inserted into the *Sma*I site in the polylinker of pORF1. As was the case for the insertion of the *amp* translation start signal [4] and as can be seen in Fig. 1, this construction resulted in the fusion of each oligonucleotide in frame with both the *ompF* and *lacZ* frames, but translation of the *lacZ* mRNA from the *ompF* start codon could be aborted by in frame stop codons designed in advance, ensuring the synthesis of β-galactosidase only from the inserted start codon (see Table II). As a control, an octadecanucleotide (ATA AGG AGG TTT AAC ATG) was also synthesized and inserted into the same site of pORF1. This oligodeoxynucleotide possesses features characteristic of a translation start signal, but its Shine-Dalgarno sequence (TAAGGAGGT) is different from that of the *amp, fusA, rpoB, glnS* and *pheT* genes (AGGA).

E. coli carrying each of the constructed plasmids was grown at 30°C to mid-log phase and then the temperature was shifted to 42°C. Before and after the temperature shift-up, cell growth and β-galactosidase activity were measured at 30-min and 5-min intervals, respectively. As shown in Fig. 2, immediately after the

Table I

E. coli genes, expression of which is transiently depressed by heat-shock, and nucleotide sequences of their translation start signal regions

Genes	Regions of the translation start signal	References
amp	ttgaaa **aAGGAA** gagt ATG	[7]
fusA	ataaac **GAGGAA** acaa ATG	[8]
glnS	cgcttt **GAGGAA** tccacg ATG	[10]
leuS	ac**agga** cAactg gctgcc ATG	[26]
pheS	cacaat **GAGGAA** aacc ATG	[11]
pheT	ataagg c**AGGAA** tagatt ATG	[11]
rpoA	caaaga **GAGGA**c aca ATG	[27]
rpoB	cgagct **GAGGAA** ccct ATG	[9]
rpsA	aaacct **GAaGA**t taaac ATG	[28]
tsf	atctcc **GAGGA**t tttag ATG	[29]
tufA	aatagt **aAGGAA** tatagcc GTG	[30]
tufB	gtctta **GAGG**gA caatcg ATG	[31]
valS	ggcaac ct**GGAA** ata ATG	[32]
argS		
aspS		
gltX		
ileS		
lysS		
Consensus sequence **GAGGAA** (N)$_{3-6}$ ATG		

The Shine-Dalgarno sequences are shown by bold letters. Consensus nucleotides appearing in this region are indicated by capital letters. A consensus sequence deduced from the listed sequences is shown in the bottom line

Table II

Oligodeoxynucleotides synthesized and inserted into the *Sma*I site of pORF1

Sources of translation start signal	Inserts
amp	**at** TAA **agg aag agt ATG**
fusA	**at** TAG **agg aaa caa ATG**
rpoB	**at** TAG **agg aac cct ATG**
glnS	**at** TAA **gag gaa tcc acc ATG**
pheT	**at** TAG **cag gaa** TAG **att ATG**
Control	**ata agg agg** ttT AAc **ATG**

The stop codon in frame with the *ompF* reading frame is indicated with capital letters. The start codon, from which the synthesis of β-galactosidase presumably initiates, is indicated with bold capital letters

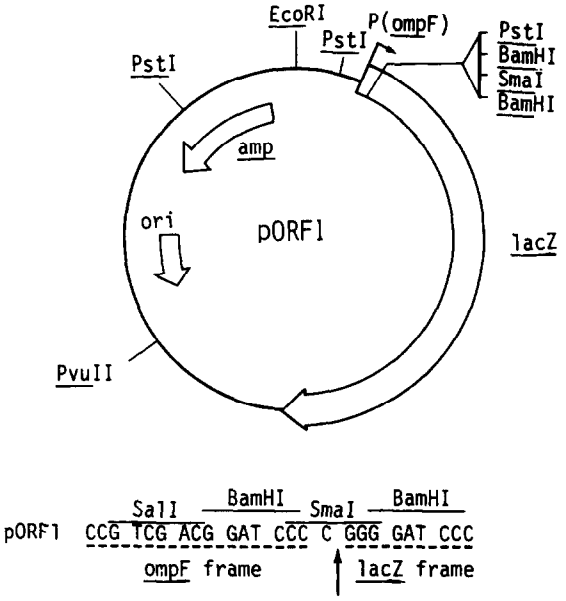

Fig. 1. The open reading frame expression vector pORF1 (top) and the nucleotide sequence of the *ompF* frame and *lacZ* frame fusion joint (bottom). The synthetic oligodeoxynucleotides shown in Table II were inserted into the *SmaI* site of pORF1, which is indicated by an arrow. The direction of transcription from the *ompF* promoter (p(*ompF*)) is also shown by an arrow on pORF1.

temperature shift-up the cell growth was elevated about 2-fold (doubling time from about 60 to about 30 min) irrespective of the plasmid introduced. In cells carrying the 'control' plasmid, an increase in β-galactosidase activity, in parallel to that of cell mass, took place upon heat shock treatment. On the other hand, in cells carrying other plasmids derived from pORF1, the *lacZ* gene of which was fused with the translation start signal of the *amp* or one of the *E. coli* genes under examination, practically no increase in β-galactosidase activity was observed for 15–20 min after the temperature shift-up. This was followed by a gradual increase in the activity to catch up with the cell growth rate at 42°C. A pulse-labeling experiment with these cells at 5 min after the temperature shift-up showed that this arrest was due to incapability of the cells to synthesize β-galactosidase (data will be published elsewhere).

Although the cellular level of β-galactosidase and the restoration pattern of its synthetic rate varied depending on the plasmid introduced into *E. coli*, the result shown in Fig. 2 indicates that at least the translation start signals of the *E. coli* genes under examination, like that of the *amp* gene [4], are involved in the heat shock-induced, transient repression of β-galactosidase synthesis. The involvement of the translation start signal

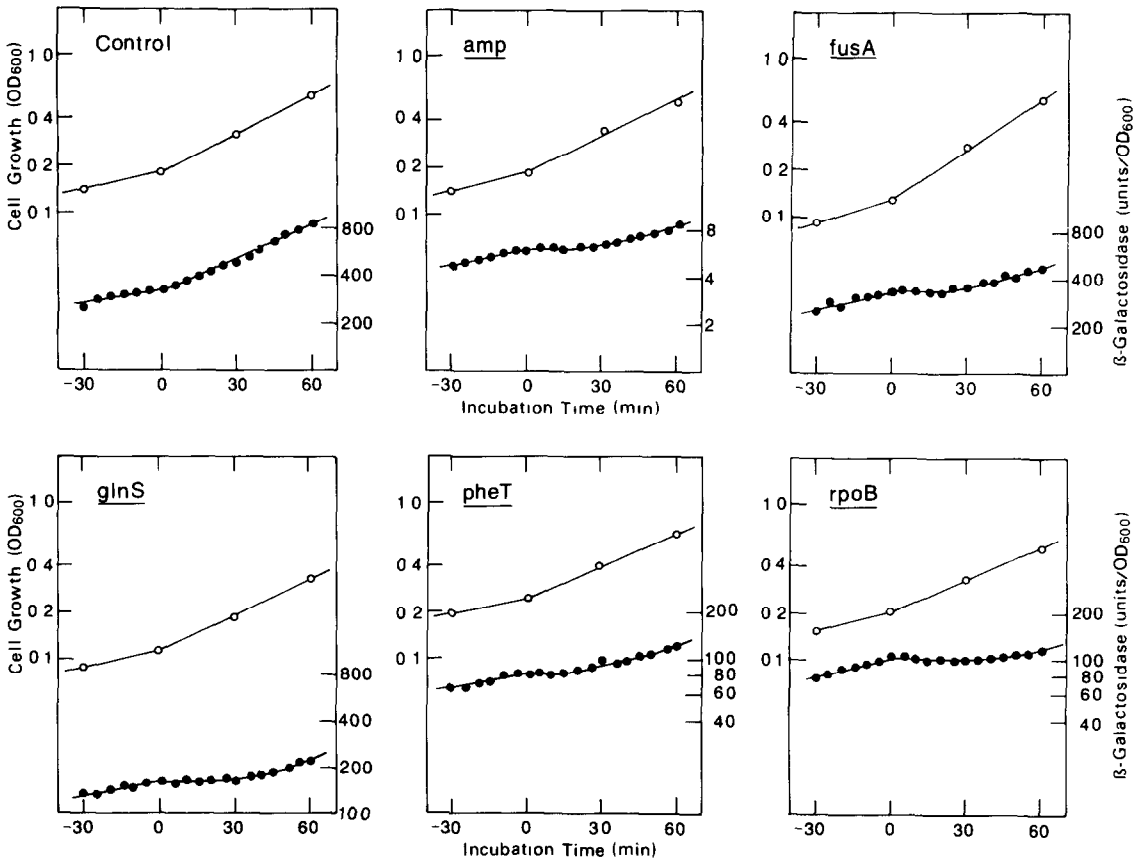

Fig. 2. Effects of temperature shift-up on growth and β-galactosidase of *E. coli* carrying the pORF1-derived plasmids. Cells harboring the constructed plasmids were grown at 30°C to mid-log phase with shaking in 50 ml of L-broth. The temperature was shifted to 42°C at time 0. Cell mass (○) was estimated from the optical density at 600 nm. β-Galactosidase activity (●) is expressed as units/ml culture.

further suggests that this arrest occurs at the initial step of translation. An interesting fact is that all of the *E. coli* genes listed in Table I encode proteins that are essential for gene expression. For example, *fusA* encodes EF-G, *rpoB* β-subunit of RNA polymerase, *glnS* glutaminyl-tRNA synthetase, and so on.

It is, however, to be noted that heat shock does not necessarily cause transient arrest of the expression of all *E. coli* genes that possess the consensus sequence GAGGAA$(N)_{3-6}$ATG at their translation start signal region. For example, the translation start signal region of the *rpoH* gene has the sequence G AGG ATT TGA ATG [17], which is almost identical with the deduced consensus sequence. Nevertheless, expression of this gene is not repressed but rather stimulated upon heat shock treatment [18–21], and this stimulation takes place not only at the transcriptional level [19,21–24] but also at the translation level [19,25]. Therefore, it is likely that, in addition to the consensus sequence deduced in this paper, another element or elements are required for heat shock induced-arrest of gene expression. Alternatively, it is possible that the ability of the translation start signal to repress translation upon heat shock is completely abolished by the flanking region(s) of the translation start signal in the *rpoH* gene. Further studies are still needed to understand the mechanism of translational repression in response to heat shock.

Acknowledgements: I thank Dr T.J. Silhavy for kindly providing the open reading frame vector pORF1, Dr R. Sato for critically reviewing the manuscript, and Dr T. Ideguchi for the synthesis of oligodeoxynucleotides, which was performed using equipment of the Center for Protein Engineering, Institute for Protein Research, Osaka University.

REFERENCES

[1] Lemaux, P.G., Herendeen, S.L., Bloch, P.L. and Neidhardt, F.C. (1978) Cell 13, 427–434.
[2] Neidhardt, F.C. and VanBogelen, R.A. (1987) in: *Escherichia coli* and *Salmonella typhimurium*. Cellular and Molecular Biology (Neidhardt, C.F., Ingraham, J.L., Low, K.B., Magasanik, B., Schaechter, M. and Umbarger, H.E. eds) pp. 1334–1345, American Society for Microbiology, Washington, DC.
[3] Kuriki, Y. (1987) J. Bacteriol. 169, 2294–2297.
[4] Kuriki, Y. (1989) Mol. Microbiol. 3, 1131–1140.
[5] Kuriki, Y. (1989) J. Bacteriol. 171, 5452–5457.
[6] Shine, J. and Dalgarno, L. (1974) Proc. Natl. Acad. Sci. USA 71, 1342–1346.
[7] Sutcliffe, J.G. (1978) Proc. Natl. Acad. Sci. USA 75, 3737–3741.
[8] Zengel, J.M., Archer, R.H. and Lindahl, L. (1984) Nucleic Acids Res. 12, 2181–2192.
[9] Post, L.E., Strycharz, G.D., Nomura, M., Lewis, H. and Denmis, P.P. (1979) Proc. Natl. Acad. Sci. USA 76, 1697–1701.
[10] Yamao, F., Inokuchi, H., Cheung, A., Ozeki, H. and Söll, D. (1982) J. Biol. Chem. 257, 11639–11643.
[11] Fayat, G., Mayaux, J., Sacerdot, C., Fromant, M., Springer, M., Grunberg-Manago, M. and Blanquet, S. (1983) J. Mol. Biol. 171, 239–261.
[12] Weinstock, G.M., Rhys, C., Berman, M.L., Hampar, B., Jackson, D., Silhavy, T.J., Weisemann, J. and Zweig, M. (1983) Proc. Natl. Acad. Sci. USA 80, 4432–4436.
[13] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[14] Sinha, N.D., Biernat, J., McManus, J. and Koester, H. (1984) Nucleic Acids Res. 12, 4539–4557.
[15] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[16] Miller, J.H. (1972) Experiments in Molecular Genetics, pp. 352–355, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[17] Yura, T., Tobe, T., Ito, K. and Osawa, T. (1984) Proc. Natl. Acad. Sci. USA 81, 6803–6807.
[18] Lesley, S.A., Thompson, N.E. and Burgess, R.R. (1987) J. Biol. Chem. 262, 5404–5407.
[19] Straus, D.B., Walter, W.A. and Gross, C.A. (1987) Nature 329, 348–351.
[20] Fujita, N., Ishihama, A., Nagasawa, Y. and Ueda, S. (1987) Mol. Gen. Genet. 210, 5–9.
[21] Tobe, T., Kusakawa, N. and Yura, T. (1987) J. Bacteriol. 169, 4128–4134.
[22] Tilly, K., Erikson, J., Sharma, S. and Georgopoulos, C. (1987) J. Bacteriol. 168, 1155–1158.
[23] Fujita, N. and Ishihama, A. (1987) Mol. Gen. Genet. 210, 10–15.
[24] Erikson, J.W., Vaughn, V., Walter, W.A., Neidhardt, F.C. and Gross, C.A. (1987) Genes Dev. 1, 419–432.
[25] Yano, R., Imai, M. and Yura, T. (1987) Mol. Gen. Genet. 207, 24–28.
[26] Härtlein, M. and Maderm, D. (1987) Nucleic Acids Res. 15, 10199–10210.
[27] Meek, D.W. and Hayward, R.S. (1984) Nucleic Acids Res. 12, 5813–5821.
[28] Schnier, J. and Isono, K. (1982) Nucleic Acids Res. 10, 1857–1865.
[29] An, G., Bendiak, D.S., Mamelak, L.A. and Friesen, J.D. (1981) Nucleic Acids Res. 9, 4163–4172.
[30] Yokota, T., Sugisaki, H., Takanami, M. and Kaziro, Y. (1980) Gene 12, 25–31.
[31] An, G. and Friesen, J.D. (1980) Gene 12, 33–39.
[32] Heck, J.D. and Hatfield, G.W. (1988) J. Biol. Chem. 263, 857–867.